

**Role Of The Reactive Site Loop In The Function Of Maspin**  
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Epithelial cells, such as those from the mammary gland, prostate, skin and cornea, synthesize maspin, a member of the serine proteinase inhibitor family of proteins. In addition, the stromal cells of the cornea synthesize this protein. Upon malignant transformation of epithelial cells to carcinoma cells or activation of corneal stromal cells to the wound healing fibroblast phenotype, synthesis of maspin is down regulated. Addition of exogenous maspin to these cells stimulates adhesion to extracellular matrix molecules and inhibits invasion. An indirect mechanism is involved probably through regulation of gene expression.

The purpose of this study was to elucidate the portion of the maspin molecule required for stimulation of adhesion of cells to extracellular matrix molecules and inhibition of invasion. Chimeras were made between the active maspin molecule and the inactive homologous serpin, ovalbumin using a yeast secretion system. Replacement of the Reactive Site Loop (RSL) or the C-terminal peptide and the RSL of maspin with that of ovalbumin resulted in the loss of stimulation of adhesion of mammary carcinoma cells to fibronectin and corneal stromal fibroblasts to fibronectin, type I collagen and laminin. These mutants also lost their ability to inhibit invasion of the carcinoma cells through a Matrigel matrix. Maspin with the C-terminal ovalbumin peptide was fully active suggesting the C-terminal region is not involved in function. Transfer of the RSL of maspin to ovalbumin resulted in conversion of ovalbumin to a fully functional molecule. The maspin RSL peptide (P10-P5', residues 330-344) alone fully stimulated adhesion and inhibited invasion. This suggests the RSL peptide is sufficient for full activity of maspin.

Site directed mutagenesis of the putative P1 Arg 339 in the RSL of intact maspin to Gln had no effect on activity but replacement of this residue with Ala resulted in the loss of activity suggesting that the side chain of Arg forms a critical hydrogen bond with its target molecule. Replacement of Lys 334 at the P5' site with Ala also resulted in the loss of activity. Substitution of the Pro 337 at the P3 site with Val had no effect but mutation to Ala resulted in the loss of activity. This suggests that the rigidity of this Pro residue is not critical for the active conformation of the RSL. These mutagenesis studies imply that multiple residues in the RSL are important for function.

Maspin bound to the surface of the mammary carcinoma cells with a  $k_d$  of  $367 \pm 67$  nM and  $32.0 \pm 2.2 \times 10^6$  binding sites/cell. This binding was inhibited by the RSL peptide suggesting the RSL is involved in the binding of maspin to cells. Sufficiency of the maspin RSL for function suggests that the mechanism by which maspin regulates cell-matrix adhesion and carcinoma cell invasion does not involve the serpin mechanism of proteinase inhibition.